

Appl. No. 10/040,077

CLAIM AMENDMENTS

1. (Original) A glucose biosensor for in vivo or in vitro use comprising:

a) at least one mutated binding protein and at least one reporter group attached thereto such that said reporter group provides a detectable and reversible signal change when said mutated binding

protein is exposed to varying glucose concentrations;

wherein said detectable and reversible signal change is related to said varying concentrations.

2. (Original) The biosensor of claim 1 wherein said mutated binding protein is glucose/galactose binding protein.

3. (Original) The biosensor of claim 1 wherein said binding protein has one amino acid substitution.

4. (Original) The biosensor of claim 1 wherein said binding protein has at least two amino acid substitutions.

5. (Original) The biosensor of claim 1 wherein said binding protein has at least three amino acid substitutions.

6. (Original) The biosensor of claim 3 wherein said one amino acid substitution is selected from the group consisting of a cysteine at position 11, a cysteine at position 14, a cysteine at position 19, a cysteine at position 43, a cysteine at position 74, a cysteine at position 107, a cysteine at position 110, a cysteine at position 112, a cysteine at position 113, a cysteine at position 137, a cysteine at position 149, a cysteine at position 213, a cysteine at position 216, a cysteine at position 238, a cysteine at position 287, and a cysteine at position 292.

7. (Original) The biosensor of claim 6 wherein said binding protein has at least one histidine tag.

8. (Original) The biosensor of claim 4 wherein said at least two amino acid substitutions are selected from the group consisting of a cysteine at position 112 and a serine at position 238, a cysteine at position 149 and a serine at position 238, a cysteine at position 152 and a cysteine at

Appl. No. 10/040,077

position 182, a cysteine at position 152 and a serine at position 213, a cysteine at position 213 and a cysteine at position 238, a cysteine at position 149 and an arginine at position 213.

9. (Original) The biosensor of claim 8 wherein said binding protein has at least one histidine tag.

10. (Original) The biosensor of claim 5 wherein said at least three amino acid substitutions are selected from the group consisting of a cysteine at position 149 and a serine at position 213 and a serine at position 238, and a cysteine at position 149 and an arginine at position 213 and a serine at position 238.

11. (Original) The biosensor of claim 10 wherein said binding protein has at least one histidine tag.

12. (Original) The biosensor of claim 1 wherein said reporter group is a luminescent label.

13. (Original) The biosensor of claim 12 wherein said luminescent label has an excitation wavelength of more than about 600 nanometers.

14. (Original) The biosensor of claim 12 wherein said luminescent label has an emission wavelength of more than about 600 nanometers.

15. (Original) The biosensor of claim 12 wherein said luminescent label is covalently coupled to said at least one glucose/galactose binding protein.

16. (Currently Amended) The biosensor of claim 15 wherein said luminescent label is covalently coupled to said at least one glucose/galactose binding protein by reaction with a member selected from the group consisting of fluorescein, coumarins, rhodamines, 5-TMR1A (tetramethylrhodamine-5-iodoacetamide), Quantum Red®, (9-(2(or4)-(N-(2-maleimidyethyl)-sulfonamidyl)-4(or 2)-sulfohenyl)-2,3,6,7,12,13,16,17-octahydro-(1H,5H,11H,15H-xantheno(2,3,4-ij:5,6,7-ij')diquinolizin-18-ium salt)-(Texas Red®), 2-(5-(1-(6-(N-(2-maleimidyethyl)-amino)-6-oxohexyl)-1,3-dihydro-3,3-dimethyl-5-sulfo-2H-indol-2-ylidene)-1,3-

Appl. No. 10/040,077

propylidienyl)-1-ethyl-3,3-dimethyl-5-sulfo-3H-indolium salt (Cy3), N-((2-iodoacetoxy)ethyl)-N-methylamino-7-nitrobenzoxadiazole (IANBD), 6-acryloyl-2-dimethylaminonaphthalene (acrylodan), pyrene, 6-amino-2,3-dihydro-2-(2-((iodoacetyl)amino)ethyl)-1,3-dioxo-1H-benz(de)isoquinoline-5,8-disulfonic acid salt (Lucifer Yellow), 2-(5-(1-(6-(N-(2-maleimidyloethyl)-amino)-6-oxohexyl)-1,3-dihydro-3,3-dimethyl-5-sulfo-2H-indol-2-ylidene)-1,3-pentadienyl)-1-ethyl-3,3-dimethyl-5-sulfo-3H-indolium salt (Cy5), DepoxyTM-4-(5-(4-dimethylaminophenyl)oxazole-2-yl)-N-(2-bromoacetamidoethyl)sulfonamide, (N-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene-2-yl)iodoacetamide (Bodipy 507/545 IA), N-(4,4-difluoro-5,7-diphenyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-N'-iodoacetylenediamine (BODIPYTM-530/550 IA), 5-((((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid (1,5-LAEDANS), and carboxy-X-rhodamine, 5/6-iodoacetamide (XRIA-5,6).

17. (Withdrawn) A method for glucose detection comprising:
 - (b) providing at least one mutated glucose/galactose binding protein and at least one reporter group attached thereto;
 - (c) exposing said mutated glucose/galactose binding protein to varying glucose concentrations;
 - (d) detecting a detectable and reversible signal change from said reporter group wherein said detectable and reversible signal change corresponds to said varying glucose concentrations.
18. (Withdrawn) The method of claim 17 wherein said detecting is continuous, programmed, episodic, or combinations thereof.
19. (Withdrawn) The method of claim 17 wherein said mutated glucose/galactose binding protein is selected from bacterial periplasmic binding proteins.
20. (Withdrawn) The method of claim 17 wherein said detecting of detectable and reversible signal changes from said reporter group of varying glucose concentrations is in vivo.

Appl. No. 10/040,077

21. (Withdrawn) The method of claim 17 wherein said binding protein has one amino acid substitution.

22. (Withdrawn) The method of claim 17 wherein said binding protein has at least two amino acid substitutions.

23. (Withdrawn) The method of claim 17 wherein said binding protein has at least three amino acid substitutions.

24. (Withdrawn) The method of claim 21 wherein said one amino acid substitution is selected from the group consisting of a cysteine at position 11, a cysteine at position 14, a cysteine at position 19, a cysteine at position 43, a cysteine at position 74, a cysteine at position 107, a cysteine at position 110, a cysteine at position 112, a cysteine at position 113, a cysteine at position 137, a cysteine at position 149, a cysteine at position 213, a cysteine at position 216, a cysteine at position 238, a cysteine at position 287, and a cysteine at position 292.

25. (Withdrawn) The method of claim 24 wherein said glucose/galactose binding protein has at least one histidine tag.

26. (Withdrawn) The method of claim 22 wherein said glucose/galactose binding protein has at least two amino acid substitutions selected from the group consisting of a cysteine at position 112 and a serine at position 238, a cysteine at position 149 and a serine at position 238, a cysteine at position 152 and a cysteine at position 182, a cysteine at position 152 and a serine at position 213, a cysteine at position 213 and a cysteine at position 238, a cysteine at position 149 and an arginine at position 213.

27. (Withdrawn) The method of claim 26 wherein said glucose/galactose binding protein has at least one histidine tag.

28. (Withdrawn) The method of claim 23 wherein said glucose/galactose binding protein has at least three amino acid substitutions selected from the group consisting of a cysteine at

Appl. No. 10/040,077

position 149 and a serine at position 213 and a serine at position 238, and a cysteine at position 149 and an arginine at position 213 and a serine at position 238.

29. (Withdrawn) The method of claim 28 wherein said glucose/galactose binding protein has at least one histidine tag.

30. (Withdrawn) The method of claim 17 wherein said at least one reporter group is a luminescent label.

31. (Withdrawn) The method of claim 30 wherein said luminescent label has an excitation wavelength of more than about 600 nanometers.

32. (Withdrawn) The method of claim 30 wherein said luminescent label has an emission wavelength of more than about 600 nanometers.

33. (Withdrawn) The method of claim 30 wherein said luminescent label is covalently coupled to said at least one glucose/galactose binding protein by reaction with said at least one mutated binding protein and a member selected from the group consisting of fluorescein, coumarins, rhodamines, 5-TMR1A (tetramethylrhodamine-5-iodoacetamide), Quantum Red™, Texas Red™, Cy3, N-((2-iodoacetoxy)ethyl)-N-methylamino-7-nitrobenzoxadiazole (IANBD), 6-acryloyl-2-dimethylaminonaphthalene (acrylodan), pyrene, Lucifer Yellow, Cy5, Dapoxyl® (2-bromoacetamidoethyl)sulfonamide, N-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene-2-yl)iodoacetamide (Bodipy507/545 IA), N-(4,4-difluoro-5,7-diphenyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-N'-iodoacetylthylenediamine (BODIPY® 530/550 IA), 5-(((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid (1,5-IAEDANS), and carboxy-X-rhodamine, 5/6-iodoacetamide (XR1A 5,6).

34. (Withdrawn) A composition comprising:
a mutated glucose/galactose binding protein having at least one amino acid substitution selected from the group consisting of a cysteine at position 11, a cysteine at position 14, a cysteine at position 19, a cysteine at position 43, a cysteine at position 74, a cysteine at position

Appl. No. 10/040,077

107, a cysteine at position 110, a cysteine at position 112, a cysteine at position 113, a cysteine at position 137, a cysteine at position 149, a cysteine at position 213, a cysteine at position 216, a cysteine at position 238, a cysteine at position 287, and a cysteine at position 292.

35. (Withdrawn) The composition of claim 34 wherein said mutated glucose/galactose binding protein has at least one histidine tag.

36. (Withdrawn) The composition of claim 34 wherein said mutated glucose/galactose binding protein further has at least one reporter group.

37. (Withdrawn) The composition of claim 36 wherein at least one reporter group is a luminescent label.

38. (Withdrawn) The composition of claim 37 wherein said luminescent label has an excitation wavelength of more than about 600 nanometers.

39. (Withdrawn) The composition of claim 37 wherein said luminescent label has an emission wavelength of more than about 600 nanometers.

40. (Withdrawn) The composition of claim 37 wherein said luminescent label is covalently coupled to said at least one glucose/galactose binding protein by reaction with said at least one mutated binding protein and a member selected from the group consisting of fluorescein, coumarins, rhodamines, 5-TMR1A (tetramethylrhodamine-5-iodoacetamide), Quantum Red™, Texas Red™, Cy3, N-((2-iodoacetoxy)ethyl)-N-methylamino-7-nitrobenzoxadiazole (IANBD), 6-acryloyl-2-dimethylaminonaphthalene (acrylodan), pyrene, Lucifer Yellow, Cy5, Dapoxyl® (2-bromoacetamidoethyl)sulfonamide, N-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene-2-yl)iodoacetamide (Bodipy507/545 IA), N-(4,4-difluoro-5,7-diphenyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-N'-iodoacetyl ethylenediamine (BODIPY® 530/550 IA), 5-(((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid (1,5-IAEDANS), and carboxy-X-rhodamine, 5/6-iodoacetamide (XR1A 5,6).

Appl. No. 10/040,077

41. (Withdrawn) A composition comprising:

a mutated glucose/galactose binding protein having at least two amino acid substitutions selected from the group consisting of a cysteine at position 112 and a serine at position 238, a cysteine at position 149 and a serine at position 238, a cysteine at position 152 and a cysteine at position 182, a cysteine at position 152 and a serine at position 213, a cysteine at position 213 and a cysteine at position 238, a cysteine at position 149 and an arginine at position 213, and a cysteine at position 149 and a serine at position 213 and a serine at position 238, and a cysteine at position 149 and an arginine at position 213 and a serine at position 238.

42. (Withdrawn) The composition of claim 41 wherein said mutated glucose/galactose binding protein has at least one histidine tag.

43. (Withdrawn) The composition of claim 41 wherein said mutated glucose/galactose binding protein further has at least one reporter group.

44. (Withdrawn) The composition of claim 43 wherein at least one reporter group is a luminescent label.

45. (Withdrawn) The composition of claim 44 wherein said luminescent label has an excitation wavelength of more than about 600 nanometers.

46. (Withdrawn) The composition of claim 44 wherein said luminescent label has an emission wavelength of more than about 600 nanometers.

47. (Withdrawn) The composition of claim 44 wherein said luminescent label is covalently coupled to said at least one glucose/galactose binding protein by reaction with said at least one mutated binding protein and a member selected from the group consisting of fluorescein, coumarins, rhodamines, 5-TMR1A Quantum Red™, Texas Red™, Cy3, N-((2-iodoacetoxy)ethyl)-N-methylamino-7-nitrobenzoxadiazole (IANBD), 6-acryloyl-2-dimethylaminonaphthalene (acrylodan), pyrene, Lucifer Yellow, Cy5, Dapoxyl® (2-bromoacetamidoethyl)sulfonamide, (N-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-

Appl. No. 10/040,077

indacene-2-yl)iodoacetamide (Bodipy507/545 IA), *N*-(4,4-difluoro-5,7-diphenyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionyl)-*N'*-iodoacetylenediamine (BODIPY® 530/550 IA), 5-(((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid (1,5-IAEDANS), and carboxy-X-rhodamine, 5/6-iodoacetamide (XRJA 5,6).
